

## Effects of Noise and Tonal Stimuli on Hearing in Pinnipeds

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### LONG-TERM GOALS

Pinnipeds (seals, sea lions, and walruses) are amphibious carnivores that are particularly susceptible to coastal anthropogenic noise impacts. The long-term goals of this effort are to improve understanding of 1) the sound detection capabilities of pinnipeds, and 2) the auditory effects of noise exposure on pinnipeds.

### OBJECTIVES

In order to examine sound detection capabilities, well established psychoacoustic methods are applied with captive, trained animals in a laboratory setting, and electrophysiological methods are developed, applied, and optimized with anesthetized animals in a rehabilitation setting. These complementary approaches are used to obtain accurate measurements of hearing sensitivity as a function of sound amplitude, sound frequency, and, when possible, transmission medium (air or water), with the aim of establishing reasonable estimates of within- and between-subject variability in auditory performance.

In order to examine the residual auditory effects of noise exposure on pinnipeds, carefully controlled laboratory experiments are conducted with highly trained animals. Hearing sensitivity is evaluated prior to and following exposure to broadband or tonal noise, and hearing thresholds are repeatedly measured over time. The specific goals of this effort are to model the onset, growth, and recovery of noise-induced temporary threshold shift (TTS) with respect to the duration, amplitude, and energy of the noise exposure; to opportunistically explore the relationship between temporary and permanent auditory threshold shifts; and to determine how hearing thresholds change permanently as a function of aging.

### APPROACH

These studies concern three representative pinniped species: northern elephant seal (*Mirounga angustirostris*), harbor seal (*Phoca vitulina*), and California sea lion (*Zalophus californianus*). At Long Marine Laboratory, hearing tests are conducted in air or under water, in a quiet hemi-anechoic

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acoustic chamber or in an acoustically mapped saltwater tank. Animals are trained for rapid measurement of hearing sensitivity using positive reinforcement and operant conditioning methods. Auditory thresholds for 500 ms tonal sounds (either pure tones or frequency modulated signals with 10% modulation bandwidth) are obtained from each subject using a psychophysical method of limits and/or a psychophysical method of constant stimuli. Threshold shifts are calculated as the difference in hearing thresholds measured just before and just after noise exposure. Noise exposures are also performed under voluntary control, with the subjects waiting at a stationing position during transmission of a fatiguing stimulus until cued to resume audiometric testing.

At The Marine Mammal Center, wild individuals of the same three species are tested while undergoing rehabilitation. Audiometric testing occurs during anesthetic procedures associated with medical treatment. Electrophysiological methods are used to observe and record auditory evoked potentials (AEPs) generated at and below the level of the brainstem. AEPs are recorded without harm to animals using subcutaneous electrodes similar to those used for ECG measurement. Signal processing techniques are used to extract, identify, and measure AEPs elicited by broadband and narrowband stimuli that are systematically varied in sound pressure level. Inter- and intra-individual assessments of variability in hearing sensitivity are conducted across a range of test frequencies. These assessments support the development of species-appropriate AEP methods for pinnipeds, with the larger sample of individuals tested serving to improve population-level estimates of hearing sensitivity.

A small team of researchers and students at Long Marine Laboratory participate in the study. Colleen Reichmuth is the Principal Investigator and oversees all research activities, animal care and use activities, and administrative requirements. She is assisted by research specialist Amy Bernard, who also serves as the lead marine mammal trainer for the project. Graduate students Jason Mulsow and Asila Ghouh participate in experimental sessions, train animals for husbandry and data collection procedures, and assist in data analysis. The team is directly supported by an experienced crew of undergraduate volunteer research assistants. Ron Schusterman of UC Santa Cruz provides assistance with psychophysical testing methodologies and James Finneran of the US Navy Marine Mammal Program contributes occasional technical expertise and software development for hearing assessment procedures.

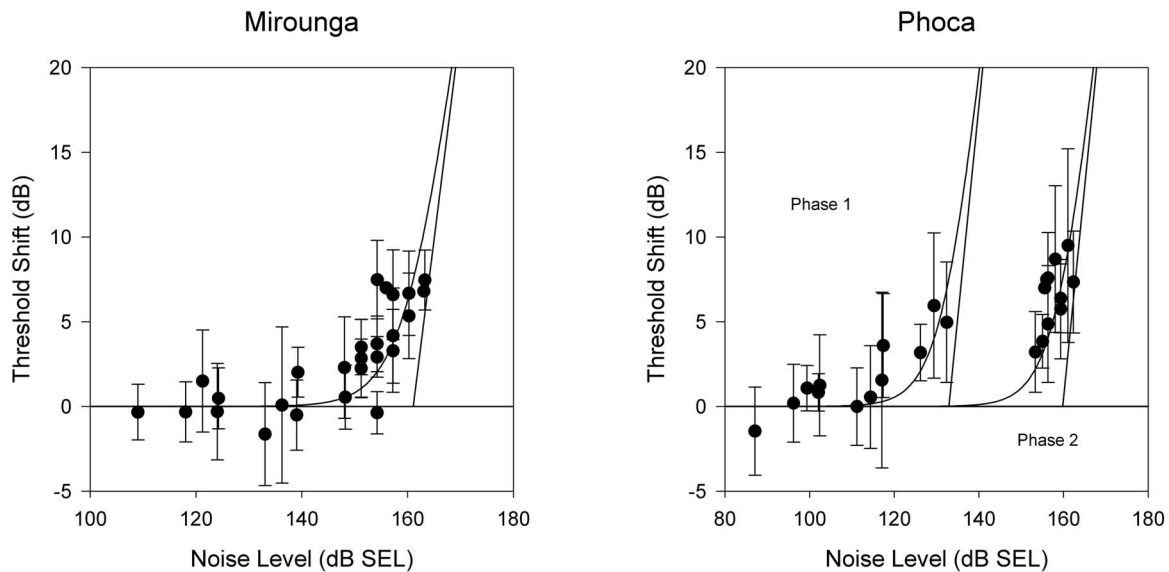
## **WORK COMPLETED**

Both psychoacoustic and electrophysiological methods were successfully applied to improve knowledge of sound detection capabilities in pinnipeds and to assess temporary and permanent changes in hearing sensitivity related to noise exposure or aging. Major accomplishments included 1) transition from examining the auditory effects of broadband noise exposure on three pinniped species to examining temporary threshold shift as a function of tonal noise exposure, 2) documentation of a permanent noise-induced threshold shift throughout a 24 month 'recovery' period, 3) assessment of natural hearing loss as a function of aging, and 4) development and application of electrophysiological methods for the assessment of aerial hearing sensitivity in pinnipeds, with a particular focus on California sea lions.

## **RESULTS**

1. Prior psychoacoustic work evaluating the effects of octave-band noise exposure on hearing sensitivity revealed predictable onset and growth of TTS as a function of noise sound pressure level

and duration. This work did not support an “equal-energy” rule but rather indicated that exposure duration had a larger influence than exposure level on determining noise-induced hearing loss. Comparative measurements of TTS made for the same individuals under identical exposure conditions showed equivalent hearing losses in air and under water. This finding supports the hypothesis that—for amphibious mammals such as pinnipeds—temporary threshold shifts that are induced in one medium result in comparable hearing losses in the other, despite differences in absolute aerial and underwater hearing sensitivity. Later testing revealed a learned protective response in the harbor seal that diminished the effects of aerial noise exposure.

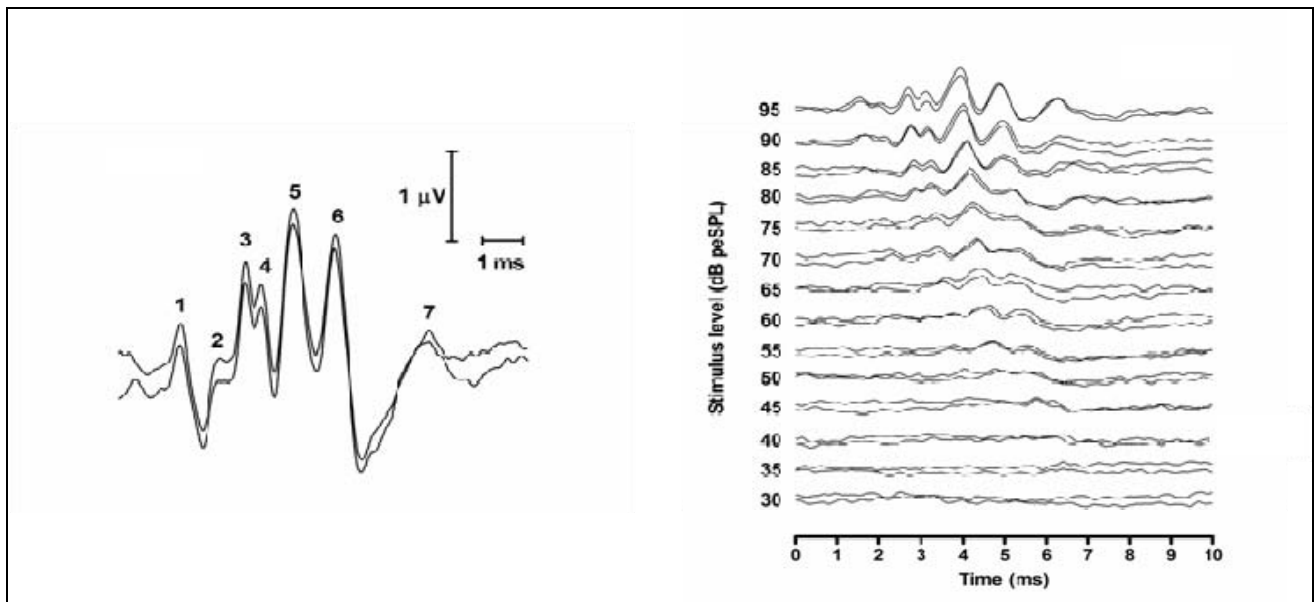


**Figure 2. The amount of TTS observed as function of noise sound exposure level in dB re (20 Pa)<sup>2</sup>s in a northern elephant seal (left) and a harbor seal (right) tested in air. Comparable findings for a California sea lion are reported in Kastak et al., 2007. The data shown for the harbor seal in Phase 2 suggests a protective mechanism related to voluntary closure of the auditory meatus.**

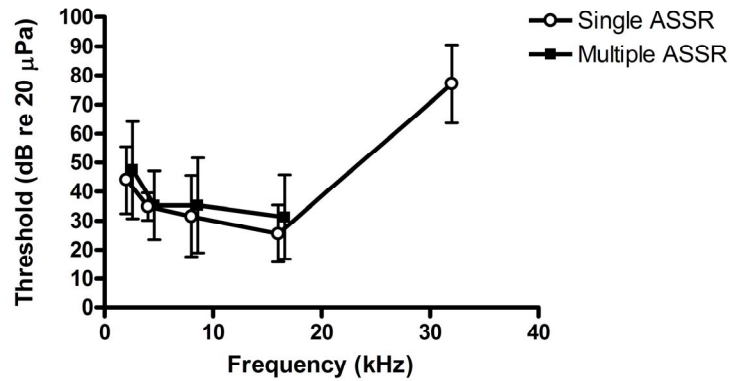
2. Temporary hearing loss induced by exposure to mid-frequency tonal noise was explored initially in a harbor seal tested under water. As the sound exposure level of the noise was gradually increased, the onset and growth of TTS did not follow the expected pattern. Rather than showing a progressive increase in TTS with increasing sound exposure level, the seal shifted suddenly from a level of no measurable effect to a dramatic threshold shift in excess of 48 dB at a frequency one half-octave above that of the tonal noise exposure. No unusual behavioral response was observed during this noise exposure condition. While hearing recovered rapidly in the hours following this occurrence, recovery was not complete, even after 24 months, resulting in a narrowband hearing loss of approximately 8 to 10 dB. This unusual event is the first case of a permanent threshold shift induced by a known noise exposure in a marine mammal, and suggests that tonal noise exposures, not commonly studied in terrestrial models of hearing (for review see Salvi and Boettcher, 2008), may be of particular concern with respect to residual auditory effects.

3. Presbycusis, or hearing loss associated with aging, was identified in two California sea lions tested repeatedly over many years. Permanent threshold shifts were observed first at higher frequencies ( $>8$  kHz) but eventually spread downward across the frequency range of hearing. Threshold shifts ranged from 0 to  $>30$  dB. Despite differences in absolute hearing sensitivity between media, the relative losses observed at each frequency were similar in air and under water. This finding confirms that permanent hearing losses induced in either medium are expressed amphibiously. Based on this and the earlier findings of TTS with broadband noise exposures, the same is likely to be true for temporary noise-induced hearing losses.

4. Appropriate electrophysiological methods for the assessment of aerial hearing sensitivity in pinnipeds were developed and refined. During this funding cycle, emphasis was placed on establishing methods for obtaining population-level measures of hearing in California sea lions. Initially, individual auditory brainstem responses (ABRs) were described and evaluated visually. ABR latency and amplitude changed predictably as a function of stimulus level. Progressive research steps led to the use of more narrowband sinusoidally amplitude-modulated stimuli that evoked relatively small but sustained or “steady-state” responses that could be evaluated statistically. These methods were then used to measure variability in hearing sensitivity from 2 to 32 kHz in a larger sample of individuals. Testing was conducted across these frequencies successively (using a single auditory steady-state response method, or single-ASSR) or simultaneously (using a multiple auditory steady-state response method, or multiple-ASSR). The relatively rapid auditory testing protocols developed for sea lions should provide a useful complement to traditional psychoacoustic methods, but at present are limited to in air applications and test frequencies greater than 1 kHz.



**Figure 3. (left) The auditory brainstem response (ABR) of a California sea lion elicited by a  $100\mu$ s click. Two traces, each derived from 500 averaged responses, are shown. (right) A series of ABRs measured as sound pressure level is decreased in 5 dB intervals. The last detectable response is at 40 dB peSPL re 20  $\mu$ Pa in this example.**



**Figure 4.** *Hearing sensitivity in seven California sea lions measured as a function of stimulus frequency. The thresholds were obtained using two complementary AEP methods (single ASSR and multiple ASSR) and provide similar results. Error bars show one standard deviation.*

## IMPACT/APPLICATIONS

The TTS data generated by this project and preceding projects have contributed to the first set of formal recommendations for noise exposure criteria developed specifically for free-ranging marine mammals, which in turn have been widely used by the operational Navy, industry, and U.S. and International regulators to establish appropriate guidelines and mitigation for anthropogenic noise emissions in marine environments.

The unanticipated results dealing with PTS generated by this project have implications relating to the use of mid-frequency sonar in coastal environments. The lack of measurable behavioral reaction or avoidance during this damaging exposure event will necessitate reconsideration of anthropogenic noise mitigation measures relying solely on behavioral disturbance metrics.

## RELATED PROJECTS

*An Opportunistic Study of Hearing in Sea Otters (*Enhydra lutris*): Measurement of Auditory Detection Thresholds for Tonal and Industry Sounds.* C. Reichmuth (UC Santa Cruz) is the PI; the project is supported by Minerals Management Service. This project expands upon auditory research with pinnipeds by examining hearing in another marine carnivore, the sea otter. There is overlap in facilities, experimental resources, and personnel.

*Detection and Tracking of Submerged Hydrodynamic Wakes Using a Bioinspired Hybrid Fluid Motion Sensor Array.* J. Humphrey (U of Virginia) is the PI; the project is supported by the Office of Naval Research. Field testing of sensor design is conducted at Long Marine Laboratory with one of the seals involved in the current project.

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